Reprinted from

Journal of Neuroimmunology

Journal of Neuroimmunology 106 (2000) 189–197

Autoimmune T cells retard the loss of function in injured rat optic nerves

Gila Moalem[a,b], Eti Yoles[a], Raya Leibowitz-Amit[a], Shir Muller-Gilor[a], Felix Mor[b], Irun R. Cohen[b], Michal Schwartz[a,*]

[a]*The Weizmann Institute of Science, Department of Neurobiology, 76100 Rehovot, Israel*
[b]*The Weizmann Institute of Science, Department of Immunology, 76100 Rehovot, Israel*

Received 11 January 2000; accepted 7 February 2000

ELSEVIER

Journal of
Neuroimmunology

Aims and Scope

The *Journal of Neuroimmunology* affords a forum for the publication of works applying immunologic methodology to the furtherance of the neurological sciences. Studies on all branches of the neurosciences, particularly fundamental and applied neurobiology, neurology, neuropathology, neurochemistry, neurovirology, neuroendocrinology, neuromuscular research, neuropharmacology and psychology, which involve either immunologic methodology (e.g. immunocytochemistry) or fundamental immunology (e.g. antibody and lymphocyte assays), are considered for publication. Works pertaining to multiple sclerosis, AIDS, amyotrophic lateral sclerosis, Guillain Barré syndrome, myasthenia gravis, and brain tumors form a major focus. The scope of the Journal is broad, covering both research and clinical problems of neuroscientific interest. A major aim of the Journal is to encourage the development of immunologic approaches to analtyse in further depth the interactions and specific properties of nervous tissue elements during development and disease.

Instructions to Authors

These instructions can also be found on the World Wide Web: access under http://www.elsevier.nl or http://www.elsevier.com
Submission of a manuscript will be held to imply that it contains original work and that it has not been published or submitted for publication elsewhere. It also implies the transfer of the Copyright from the author to the publisher. Papers and original illustrations should be submitted plus two copies and accompanied by a letter of transmittal to the Editor-in-Chief or to the Associate Editor for Clinical Neuroimmunology. After final acceptance of an article only corrections to the title and list of authors as they appear on the accepted article will be permitted, not changes; particularly, no author names may be added to or deleted from the accepted lists.

Types of Papers

The journal primarily publishes full length papers describing original work; but also includes Review articles. Review articles will normally be by invitation and individuals wishing to submit a Review should first take up contact with the Editor. Short communications, not exceeding 1500 words, will be accepted for rapid publication.

Preparation of Manuscripts

The **preferred** medium of final submission to the accepting editor is on disk with accompanying reviewed and revised manuscript (see 'Electronic manuscripts' below).
Manuscripts should be in the English language and should be typewritten *with double spacing* on one side of the paper only. A short Abstract, not exceeding 100 words, should be on a separate sheet; it will appear at the beginning of the paper. A list of 3–6 key words should appear underneath the Abstract. The title page should be a separate sheet, bearing title, author(s)' name(s) and their *full* addresses, and a footnote with the corresponding author's Telephone, Fax numbers and E-mail address. As a rule, papers should be divided into numbered sections, headed by a caption (e.g. Abstract; 1. Introduction; 2. Materials and methods; 3. Results; 4. Discussion; References). **Authors in Japan please note:** Upon request, Elsevier Science K.K. will provide authors with a list of people who can check and improve the English of their paper (*before submission*). Please contact our Tokyo office: Elsevier Science K.K. 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106-0044; Japan; Tel.: +81-3-55615033; Fax: +81-3-55615045; E-mail: info@elsevier.co.jp

Acknowledgement: The cover illustration shows an astrocyte identifiable by positive immunocytochemistry to glial fibrillary acidic protein (GFAP), courtesy of Prof. S. Rotshenker, Hebrew University-Hadassah Medical School, Jerusalem (J. Neuroimmunol., 70 (1996) p. 158).

(continued on inside back cover)

ELSEVI

Autoi

Gila Mo

Abstract

We rece
axotomy ir
manifested
protein sig
response e
and sugge
slow dow

Keywords:

1. Intro

Axona
(CNS) i
injury sit
to regro
correspo
addition,
located i
adequate
(Faden,
Schwart
results f
and con
Schwart
that mig
are bio
glucose
free rac
endoger
al., 199
1993; L

*Corre
4131.
E-mail

0165-572
PII: S0

EVIER

Journal of Neuroimmunology 106 (2000) 189–197

Journal of
Neuroimmunology

www.elsevier.com/locate/jneuroin

oimmune T cells retard the loss of function in injured rat optic nerves

Moalem[a,b], Eti Yoles[a], Raya Leibowitz-Amit[a], Shir Muller-Gilor[a], Felix Mor[b], Irun R. Cohen[b], Michal Schwartz[a,*]

[a]*The Weizmann Institute of Science, Department of Neurobiology, 76100 Rehovot, Israel*
[b]*The Weizmann Institute of Science, Department of Immunology, 76100 Rehovot, Israel*

Received 11 January 2000; accepted 7 February 2000

t

cently demonstrated that autoimmune T cells protect neurons from secondary degeneration after central nervous system (CNS) in rats. Here we show, using both morphological and electrophysiological analyses, that the neuroprotection is long-lasting and is ed functionally. After partial crush injury of the rat optic nerve, systemic injection of autoimmune T cells specific to myelin basic ignificantly diminished the loss of retinal ganglion cells and conducting axons, and significantly retarded the loss of the visual evoked by light stimulation. These results support our challenge to the traditional concept of autoimmunity as always harmful, est that in certain situations T cell autoimmunity may actually be beneficial. It might be possible to employ T cell intervention to /n functional loss in the injured CNS. © 2000 Published by Elsevier Science B.V. All rights reserved.

Autoimmunity; CNS; Injury

duction

l injury in the mammalian central nervous system nitiates a process of axonal degeneration at the e, usually leading to failure of the damaged fibers v and reconnect, and the eventual death of the ıding cell bodies (Ramon y Cajal, 1959). In fibers that have not sustained direct injury but are ı the vicinity of the injured neurons will, unless y treated, undergo secondary degeneration 1993; Lynch and Dawson, 1994; Yoles and 1998). This progressive spread of damage ım a process that begins within minutes of injury ıues for days or weeks (Faden, 1993; Yoles and 1998). Among the injury-related mechanisms t underlie the post-traumatic spread of damage emical and metabolic changes in oxygen and ilization, energy state, lipid-dependent enzymes, als, eicosanoids, tissue ions, biogenic amines, ıs opioids, and excitatory amino acids (Hovda et Yoshino et al., 1991; Yoles et al., 1992; Faden, et al., 1994). These changes cause alterations in cellular homeostasis, excitotoxicity, local production of agents harmful to nerve cells, and a loss of trophic support from targets, all of which result in secondary neuronal loss. The proposed mechanisms of secondary degeneration have served as a basis for the development and evaluation of various pharmacological interventions for the treatment of CNS injuries. The therapeutic approach of preventing or diminishing the secondary degeneration accompanying CNS trauma is termed neuroprotection (Faden and Salzman, 1992; McIntosh, 1993; Smith et al., 1995).

T cells are important players in the adaptive arm of the immune system. T cells respond to specific antigens through interactions of their specific antigen receptor with the antigen presented by major histocompatibility complex molecules and a group of costimulatory molecules. When activated, they can kill their target cells or produce cytokines that activate or suppress the growth, movement, or differentiation of other cells. Thus, T cells play a critical part in the protection of tissues against foreign invaders as well as in tissue maintenance.

Immune responses in the CNS are relatively restricted, resulting in the status of the CNS as an immune-privileged site (Streilein, 1995). The unique nature of the communication between the CNS and the immune system can be observed, for example, in the dialog between the CNS and T cells. In the CNS, under normal conditions activated T

ıding author. Tel.: +972-8-934-2467; fax: +972-8-934-

ress: bnschwar@weizmann.weizmann.ac.il (M. Schwartz)

)/$ – see front matter
-5728(00)00240-X

cells can cross the blood–brain barrier and enter the CNS parenchyma. However, only T cells capable of reacting with a CNS antigen seem to persist there (Hickey et al., 1991). Comparative studies of the T cell response at sites of axotomy in the CNS and the peripheral nervous system (PNS), using T cell immunocytochemistry, revealed a significantly greater accumulation of endogenous T cells in the injured PNS axons than in the injured CNS axons (Moalem et al., 1999b). Moreover, in cases of inflammation, the CNS showed a high potential for elimination of T cells via apoptosis, whereas such elimination was less effective in the PNS, and was almost absent in other tissues such as muscle and skin (Gold et al., 1997). These findings, suggesting that the T cell response to CNS injury is relatively limited, prompted us to examine how augmentation of the T cell response at a site of CNS injury affects the outcome of secondary degeneration.

We recently demonstrated that systemic injection of activated T cells of different antigen specificities immediately after rat optic nerve injury (a model for CNS white matter trauma) results in an increased T cell accumulation at the injury site (Hirschberg et al., 1998; Moalem et al., 1999a). Injection of activated T cells specific to a CNS self antigen, myelin basic protein (MBP), but not to non-CNS antigens, reduced the secondary degeneration of neurons after crush injury of CNS axons (Moalem et al., 1999a). In the present study, we used morphological and electrophysiological techniques to determine the longevity of the induced neuroprotective effect. In addition, measurement of the visual evoked potential (VEP) response, reflecting the functional activity of the visual system in response to a flashing light stimulus (Spekreijse and Apkarian, 1986), was used to determine whether and to what extent the neuroprotective effect is manifested functionally in individual animals over time. We show that autoimmune T cells specific to MBP can slow down the post-traumatic functional loss of the visual response to light in injured optic nerves of adult rats, thereby providing long-lasting neuroprotection.

2. Materials and methods

2.1. Animals

Inbred female Lewis rats (8–12 weeks old) were supplied by the Animal Breeding Center of The Weizmann Institute of Science. The rats were housed in a light- and temperature-controlled room and matched for age in each experiment.

2.2. Antigens

MBP from the spinal cords of guinea pigs was prepared as described (Hirshfeld et al., 1970). The peptide 277 (p277) of the human 60-kDa heat shock protein (hsp60) (sequence VLGGGCALLRCPALDSLTPANED) (Elias et al., 1991) was synthesized using the 9-fluorenylmethoxycarbonyl technique with an automatic multiple peptide synthesizer (AMS 422, ABIMED, Langenfeld, Germany). The purity of the peptide was analyzed by HPLC and amino acid composition.

2.3. T cell lines

T cell lines were generated from draining lymph node cells obtained from Lewis rats immunized with the above antigens (Ben Nun et al., 1981). The antigen was dissolved in phosphate-buffered saline (PBS) (1 mg/ml) and emulsified with an equal volume of incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA) supplemented with 4 mg/ml of *Mycobacterium tuberculosis* (Difco). Ten days after the antigen was injected into the rats' hind foot pads in 0.1 ml of the emulsion, the rats were killed and the draining lymph nodes were surgically removed and dissociated. The cells were washed and activated with the antigen (10 μg/ml) in proliferation medium containing Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine (2 mM), 2-mercaptoethanol (5×10^{5} M), sodium pyruvate (1 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml), nonessential amino acids (1 ml/100 ml) and autologous serum 1% (v/v). After incubation for 72 h at 37°C, 90% relative humidity and 7% CO_2, the cells were transferred to propagation medium consisting of DMEM, L-glutamine, 2-mercaptoethanol, sodium pyruvate, nonessential amino acids and antibiotics in the same concentrations as above, with the addition of 10% fetal calf serum (volume/volume) and 10% T-cell growth factor derived from the supernatant of concanavalin A-stimulated splenocytes. Cells were grown in propagation medium for 4–10 days before being restimulated with their antigen (10 μg/ml) in the presence of irradiated (2000 rad) thymus cells (10^{7} cells/ml) in proliferation medium. The T cell lines were expanded by repeated stimulation and propagation (Ben Nun and Cohen, 1982).

2.4. Crush injury of optic nerve

The optic nerve was subjected to crush injury as previously described (Duvdevani et al., 1990). Briefly, rats were deeply anesthetized by intraperitoneal (i.p.) injection of Rompun (xylazine, 10 mg/kg; Vitamed, Israel) and Vetalar (ketamine, 50 mg/kg; Fort Dodge Laboratories, Fort Dodge, IA, USA). Using a binocular operating microscope, lateral canthotomy was performed in the right eye, and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was exposed intraorbitally by blunt dissection. Using calibrated cross-action forceps, the optic nerve was subjected to a crush injury 1–2 mm from the eye. Moderate crush injury was used for short-term trials (2 weeks) and

mild crush injury for long-term trials (4 weeks), as these time periods were shown to be optimal for demonstrating secondary degeneration and its response to treatment (Yoles and Schwartz, 1998). The uninjured contralateral nerve was left undisturbed.

2.5. Measurement of secondary degeneration by retrograde labeling of retinal ganglion cells

Secondary degeneration of the optic nerve axons and their attached retinal ganglion cells (RGCs) was measured after post-injury application of the fluorescent lipophilic dye, 4-(4-(didecylamino)styryl)-*N*-methylpyridinium iodide (4-Di-10-Asp) (Molecular Probes Europe BV, The Netherlands), distally to the lesion site, 4 weeks after crush injury. Because only axons that are intact can transport the dye back to their cell bodies, application of the dye distally to the lesion site after 4 weeks ensures that only axons that survived both the primary damage and the secondary degeneration will be counted. This approach enabled us to differentiate between neurons that are still functionally intact and neurons in which the axons are injured but the cell bodies are still viable, because only those neurons whose fibers are morphologically intact can take up dye applied distally to the site of injury and transport it to their cell bodies. Using this method, the number of labeled RGCs reliably reflects the number of still-functioning neurons. Labeling and measurement were carried out as follows: the right optic nerve was exposed for the second time, again without damaging the retinal blood supply. Complete axotomy was performed 1–2 mm from the distal border of the injury site and solid crystals (0.2–0.4 mm diameter) of 4-Di-10-Asp were deposited at the site of the newly formed axotomy. Five days after dye application the rats were killed. The retina was detached from the eye, prepared as a flattened whole mount in 4% paraformaldehyde solution, and examined for labeled RGCs by fluorescence microscopy.

2.6. Recording of compound action potential

Nerves were excised and their compound action potentials (CAPs) recorded in vitro using a suction electrode experimental setup (Yoles et al., 1996). Four weeks after injury and injection of T cells or PBS, rats were killed by i.p. injection of pentobarbitone (170 mg/kg) (CTS Chemical Industries, Israel). Both optic nerves were removed while still attached to the optic chiasma, and immediately transferred to a vial containing a fresh salt solution consisting of NaCl 126 mM, KCl 3 mM, NaH_2PO_4 1.25 mM, $NaHCO_3$ 26 mM, $MgSO_4$ 2 mM, $CaCl_2$ 2 mM, and D-glucose 10 mM, aerated with 95% O_2 and 5% CO_2 at room temperature. After 1 h, CAPs were recorded. In the injured nerve, recordings were obtained from a segment distal to the injury site. This segment presumably contained axons of viable RGCs that had escaped both primary and secondary damage, as well as the distal stumps of non-viable RGCs that had not yet undergone Wallerian degeneration. The nerve ends were connected to two suction Ag–AgCl electrodes immersed in the bathing solution at 37°C. A stimulating pulse was applied through the electrode, and the CAP was recorded by the distal electrode. A stimulator (SD9, Grass Medical Instruments, Quincy, MA, USA) was used for supramaximal electrical stimulation at a rate of 1 pps to ensure stimulation of all conducting axons in the nerve. The measured signal was transmitted to a microelectrode AC amplifier (model 1800, A–M Systems, Everett, WA, USA). The data were processed using the LabView 2.1.1 data acquisition and management system (National Instruments, Austin, TX, USA). For each nerve, the difference between the peak amplitude and the mean plateau of eight CAPs was computed and was considered as proportional to the number of conducting axons in the optic nerve. The experiments were performed by observers blinded to the treatment received by the rats. In each experiment the data were normalized relative to the mean CAP of the nerves from PBS-injected rats.

2.7. Recording of visual evoked potential response

Rats were anesthetized and placed in a small stereotaxis instrument. In the skull of each rat three holes were drilled, through which two electrodes were implanted in the primary visual cortices (V1) and one electrode was implanted in the nasal bone, while the dura was kept intact to minimize cortical damage. The electrodes were gold contact pins (Wire-Pro, Salem, NJ, USA) soldered to stainless steel screws, which were screwed into the holes and cemented to the skull with acrylic cement. The electrode inserted through the hole drilled in the nasal bone was used as a reference point. The other two holes were in area OC1 with coordinates bregma 8 mm and lateral 3 mm. The field potential in each eye, recorded in the contralateral visual cortex, was evoked by stroboscopic light stimulation after implantation of the electrodes. The stroboscopic light had the following characteristics: xenon flash tube (4 W/s, 1–2 ms duration, 0.3 Hz) amplified 1000 times (AM Systems, microelectrode AC amplifier, model 1800) and digitized (12 bits, 5000 samples per second) by the use of an NB-MIO 16-9 board (National Instruments) and LABVIEW 2.2.1 data acquisition and analysis software. Before and at different times after a right optic nerve crush injury and concomitant i.p. injection of T cells or PBS, VEP responses were recorded from the two primary visual cortices in each animal. The left visual cortex, which is contralateral to the injured nerve, receives most of its input from the crushed nerve, and the right visual cortex, contralateral to the uninjured nerve, receives most of its input from the intact nerve. During each measurement, the eye on the same side as the cortex from which the response was recorded was covered with black tape to eliminate the

minor contribution of that eye to the response. The VEP data were computed and the pattern of the stimulus-specific field potentials (obtained from the average of three recordings, each representing the mean response to 60 light stimuli) was compared to the pattern of the non-stimulus-specific field potential (obtained from the average of three recordings in the absence of a light stimulus) in each case. The field potentials of T cell-injected rats and PBS-injected rats were compared in order to determine the effects of the treatment on visual system integrity obtained from the left visual cortex contralateral to the injured nerve, and from the right (control) visual cortex contralateral to the intact nerve. The VEP amplitude was calculated as the voltage difference between the first negative peak of the field potential and the subsequent positive peak. Latency was calculated as the time to the first negative peak. The effects of the two treatments on VEP amplitudes and latencies over time were analyzed using the repeated measures analysis of covariance (ANCOVA), including treatment and individual rat (random effect) as nominal effects and day as a covariate. Differences in the relative effects of the two treatments over time were detected by including a treatment×day interaction term (i.e., by determining whether the linear effects of day have different slopes for the two treatments). Normality of each data set was achieved by natural logarithmic transformation. The proportion of rats with negative VEP responses in each treatment group was calculated for each time point. Differences between the two treatments over time were examined using logistic regression. Significance was tested using likelihood-ratio Chi square. All analyses were carried out using JMP (SAS Institute 1995, Cary, NC, USA).

3. Results

3.1. Autoimmune anti-MBP T cells reduce the loss of retinal ganglion cell survival in injured optic nerves

We have already demonstrated morphologically that anti-MBP T cells, injected immediately after optic nerve crush injury, protect neurons from secondary degeneration, as measured 2 weeks later (Moalem et al., 1999a). To determine whether this neuroprotective effect can also be seen 4 weeks after injury, rats were injected i.p., immediately after mild optic nerve crush injury, with 10^7 activated T cells specific to MBP. As controls, rats were injected either with 10^7 activated T cells specific to p277 of hsp60 or with PBS. The anti-MBP T cells induced the transient monophasic paralytic disease known as experimental autoimmune encephalomyelitis (EAE), which started on day 4 after cell injection, peaked on day 6 and terminated around day 10. Secondary degeneration of the optic nerve axons and their attached RGCs was measured by retrograde labeling 4 weeks after the primary injury and counting the labeled RGCs (reflecting still-viable axons) in each retina. The mean number of surviving RGCs was significantly greater in the retinas of rats injected with anti-MBP T cells than in the retinas of rats injected with anti-p277 T cells or with PBS (Fig. 1). In contrast, the mean number of surviving RGCs in the retinas of rats injected with anti-p277 T cells did not differ significantly from that in the retinas of PBS-injected rats.

3.2. Autoimmune anti-MBP T cells reduce the loss of axonal conduction in injured optic nerves

To confirm the long-lasting neuroprotective effect of the anti-MBP T cells, we carried out electrophysiological studies using CAP as a measure of nerve conduction. Immediately after mild optic nerve crush injury, rats were injected i.p. with PBS or with 10^7 activated anti-MBP T cells. The optic nerves were excised 4 weeks later and CAPs were recorded from the uninjured nerves and from the distal segments of the injured nerves. The mean CAP amplitude recorded from the distal segments of the injured nerves of rats injected with the anti-MBP T cells was

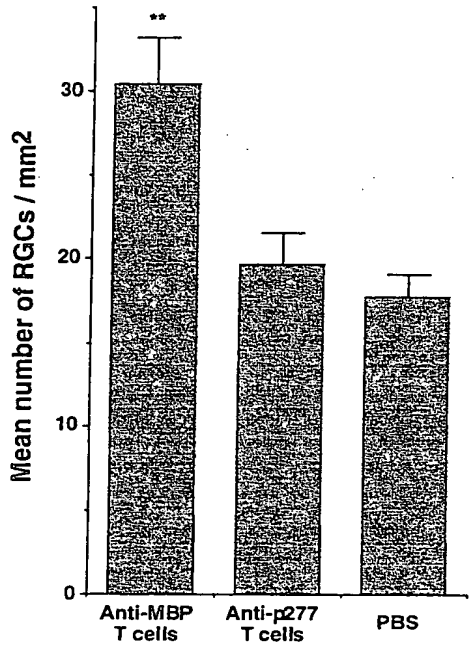

Fig. 1. Anti-MBP T cells diminish the loss of RGCs after optic nerve injury. Immediately after mild optic nerve injury rats were injected with anti-MBP T cells, anti-p277 T cells, or PBS. Four weeks after injury, the neurotracer dye 4-Di-10-Asp was applied to optic nerves distal to the injury site. Five days after dye application the retinas were excised and flat-mounted. Labeled RGCs from five randomly selected fields in each retina (all located at approximately the same distance from the optic disk) were counted by fluorescence microscopy, and their average number per mm^2 was calculated. The histogram shows the mean number of RGCs±S.E. Each group contained six to ten rats. The neuroprotective effect of the anti-MBP T cells compared with that of PBS or of the anti-p277 T cells was significant (** $P<0.01$, one-way ANOVA followed by Bonferroni's multiple comparison t-test).

significantly greater than that recorded from the PBS-injected control rats (Fig. 2). No effect of the injected anti-MBP T cells on the mean CAP amplitude was observed in uninjured nerves. These results indicate that autoimmune anti-MBP T cells produce long-term neuroprotection after CNS axonal injury.

3.3. Loss of visual evoked potential response to light in injured optic nerves is retarded after administration of anti-MBP T cells

For functional analysis of the effect of anti-MBP T cells on optic nerve degeneration, rats with electrodes implanted at the visual cortex were injected i.p., immediately after moderate unilateral optic nerve crush injury (right side), with PBS or with 10^7 activated anti-MBP T cells. VEP responses to light, previously shown to be a reliable measure of the integrity of the visual system from the retina to the cortex (Spekreijse and Apkarian, 1986; Dorfman et al., 1987), were recorded before injury and 1, 5, 7, and 15 days after injury, from both injured and uninjured nerves of rats injected with anti-MBP T cells or with PBS. The VEP responses were recorded during repetitive visual stimulation (flashing light) and showed separate components corresponding to electrical activity at each relay of the visual pathway from the eye to the cortex. The results were compared and analyzed for the potentials evoked by the left visual cortex receiving information mainly from the injured nerve and the right visual cortex (control) receiving information mainly from the uninjured nerve, before and at different time points after injury. Representative VEP responses recorded from the left visual cortex, before and after optic nerve injury in a rat injected with anti-MBP T cells or with PBS, are shown in Fig. 3.

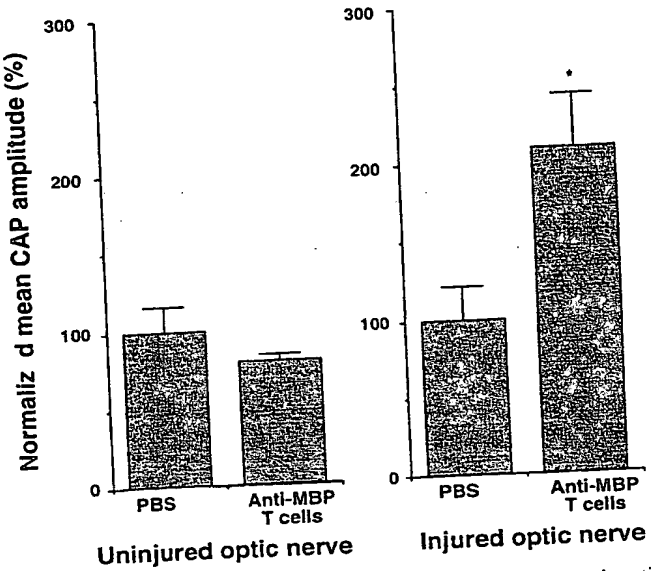

Fig. 2. Anti-MBP T cells diminish the loss of CAP in injured optic nerves. Immediately after mild optic nerve injury, rats were injected with either PBS or activated anti-MBP T cells. Four weeks later the CAPs of injured and uninjured nerves were recorded. Results were normalized by calculating the ratio between the mean CAP amplitude of nerves from T cell-injected rats and from PBS-injected rats. The histogram shows the normalized mean CAP amplitudes (%)±S.E. Each group contained six rats. There were no significant differences in mean CAP amplitudes between uninjured nerves obtained from PBS-injected rats and from T cell-injected rats. However, the neuroprotective effect of the anti-MBP T cells (relative to PBS) on the injured nerve on day 28 after injury was significant (* $P<0.05$, Student's t-test).

In rats with positive VEP responses, the latencies and amplitudes were analyzed, and the percentage of rats with negative VEP responses in each group was calculated and analyzed separately. A significantly slower decline of VEP amplitude over time, manifested by a less steep slope, was observed in the injured nerves of rats injected with anti-MBP T cells than in the injured nerves of rats injected with PBS (Fig. 4A), indicating that the autoimmune anti-MBP T cells slow down the loss of the visual response to light. In contrast, there was no significant difference in VEP amplitude over time between the uninjured nerves of the two groups of rats (Fig. 4B). The latency of the response peaks did not differ significantly either in the injured or in the uninjured nerves of the rats injected with anti-MBP T cells compared with the PBS-injected rats (Fig. 4C, D). For both treatments, the probability of a negative VEP response increased over time. However, the percentage of rats that showed negative VEP responses from the injured nerve was higher in PBS-injected rats (78% on day 15) than in rats injected with anti-MBP T cells (50% on day 15), though the difference was not significant (Fig. 4E). No negative VEP responses were obtained from the uninjured nerves in either of the groups. These results indicate that the autoimmune anti-MBP T cells retard the loss of function in injured rat optic nerves.

4. Discussion

The CNS is an immune-privileged site, in which immune activity is restricted (Streilein, 1995). Since immune responses are essential for tissue protection and repair, immune privilege might limit the ability of the CNS to defend itself against trauma. Whereas damage to peripheral tissues is followed by functional recovery, damage to CNS tissue is followed by the degeneration of directly damaged neurons, as well as the progressive secondary degeneration of neurons that escaped the primary injury (Ramon y Cajal, 1959; Faden, 1993; Yoles and Schwartz, 1998). We recently showed that systemic administration of activated T cells specific to a self-antigen, MBP, is beneficial in reducing the spread of damage following CNS axotomy in rats (Moalem et al., 1999a). In the present study we demonstrated that the neuroprotective effect exerted by the autoimmune anti-MBP T cells is long-lasting, being detectable for at least 1 month after optic nerve crush injury in the rat, and is manifested both morphologically and

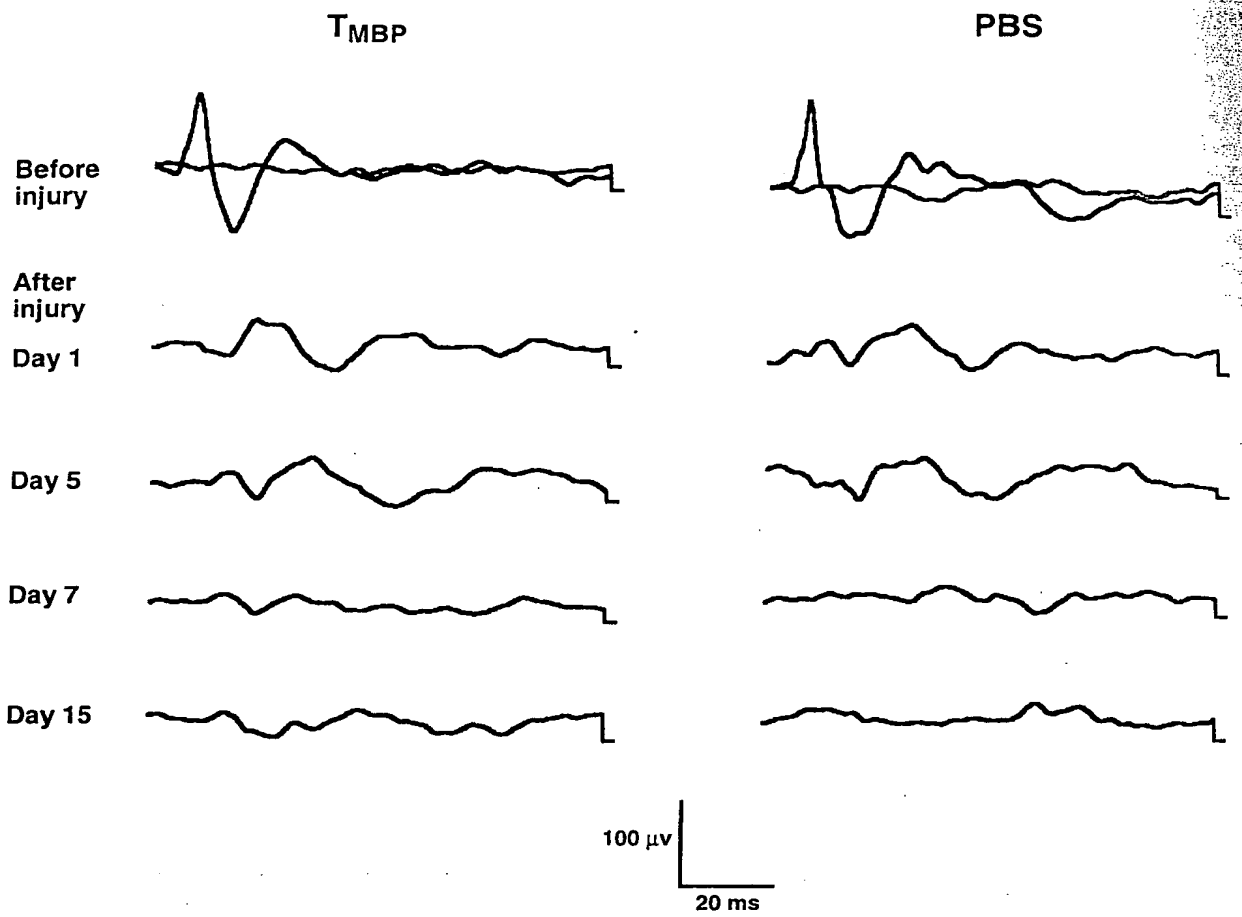

Fig. 3. Representative VEP responses to light after optic nerve injury. Immediately after moderate optic nerve injury, rats with electrodes implanted in the visual cortex were injected i.p. with PBS or with activated anti-MBP T cells (T_{MBP}). VEP responses to light were recorded before and 1, 5, 7, and 15 days after injury, from both injured and uninjured nerves. The figure shows representative VEP responses recorded from injured optic nerves of rats injected with T cells or with PBS. The grey line represents the VEP response in the absence of a light stimulus. For clarity, it is shown only at the VEP response recorded before the optic nerve injury. Note the reduction in VEP amplitude over time, and the differences in VEP responses between a rat injected with T cells and a PBS-injected rat on days 7 and 15 after injury.

electrophysiologically. The integrity of the visual system was significantly less affected by secondary degeneration in rats injected with anti-MBP T cells than in control PBS-injected rats, thus demonstrating a functional neuroprotective effect of autoimmune anti-MBP T cells in the injured rat optic nerve.

We used three independent and complementary approaches — morphological measurement, and electrophysiological recording of CAP and of VEP — to study the effect of autoimmune T cells on the outcome of optic nerve injury in the rat. The morphological result was achieved by direct counting of retrogradely labeled RGCs, yielding the number of axons that were still viable. The CAP assessment is a direct in vitro measurement of the propagative properties of a bundle of axons; a higher CAP amplitude signifies a larger number of conducting axons (Stys et al., 1991). The CAP is recorded from a segment of the injured nerve that contains both axons that escaped the primary insult and injured axons that have not yet degenerated. Therefore, a higher CAP amplitude could reflect the rescue of spared neurons, or a delay of Wallerian degeneration of injured axons, or both. In this study, the results of the CAP measurement verified the morphological analysis showing that the neuroprotective effect of the autoimmune anti-MBP T cells lasts for at least 4 weeks after optic nerve injury.

Unlike the morphological and CAP measurements, which are performed at a single time point in each rat, measurement of VEP enables us to carry out a non-invasive follow-up of the same rat over time. The VEP does not measure directly the number of RGCs or the number of axons in the optic nerve, but assesses the overall activity of cortical neurons in response to a flashing light stimulus (Spekreijse and Apkarian, 1986; Dorfman et al., 1987). The potentials evoked by the light originate in the retina and are propagated along the axons to reach their final target, the visual cortex. The visual activity is affected by crush injury of the optic nerve and the consequent

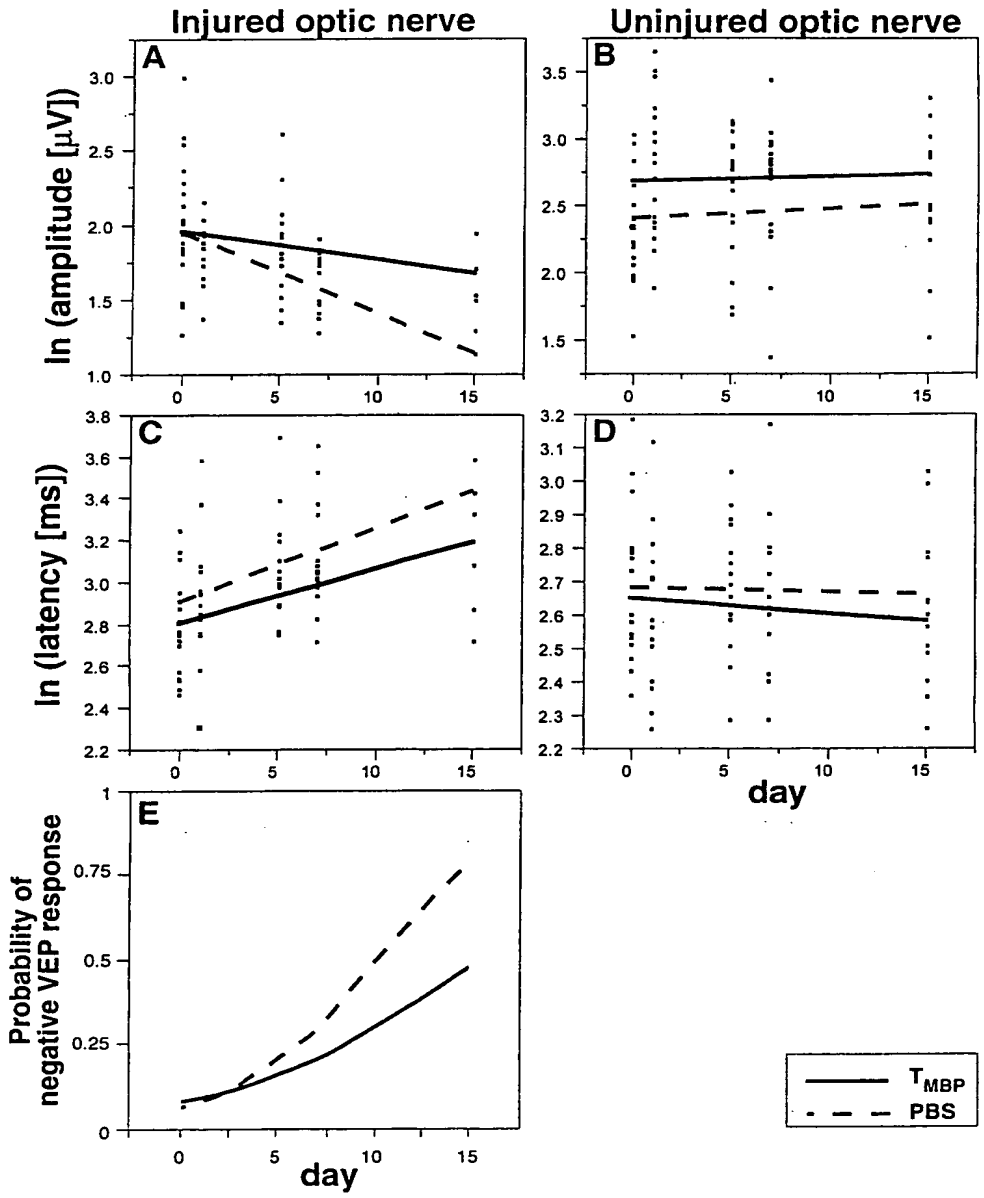

Fig. 4. Anti-MBP T cells slow down the loss of VEP response to light after optic nerve injury. Immediately after moderate optic nerve injury, rats with electrodes implanted in the visual cortex were injected i.p. with PBS or with activated anti-MBP T cells (T_{MBP}). VEP responses to light were recorded before and 1, 5, 7, and 15 days after injury, from both injured and uninjured nerves. The computed results were analyzed for the effects of the two treatments on VEP amplitudes and latencies over time and for the probability of negative VEP responses over time. Each group contained nine or ten rats. (A) VEP amplitudes (natural logarithmic transformed) from the visual cortices contralateral to the injured nerves. The slope of VEP amplitude over time in the anti-MBP T cell treatment was significantly less steep than that obtained with PBS treatment ($P=0.029$, ANCOVA). (B) VEP amplitudes (natural logarithmic transformed) from the visual cortices contralateral to the uninjured nerves. There was no significant difference in VEP amplitude over time between the two treatments ($P=0.702$, ANCOVA). (C) VEP latencies (natural logarithmic transformed) from the visual cortices contralateral to the injured nerves. There was no significant difference in VEP latency over time between the two treatments ($P=0.482$, ANCOVA). (D) VEP latencies (natural logarithmic transformed) from the visual cortices contralateral to the uninjured nerves. There was no significant difference in VEP latency over time between the two treatments ($P=0.803$, ANCOVA). (E) Probability of negative VEP responses. There was no significant difference in recorded negative VEP responses between the two treatments ($P=0.3$, Chi square test). Notably, however, there was a higher proportion of negative VEP responses in the PBS-treated rats than in the rats treated with anti-MBP T cells.

secondary degeneration. Only axons that have survived both the primary and the secondary degenerative processes are capable of conducting action potentials. Thus, a comparison of the VEP amplitude between the rats injected with T cells and the PBS-injected rats reveals the effect of treatment on the integrity of the visual pathway as a whole.

The smaller decline in VEP amplitude observed in rats subjected to optic nerve crush injury and injection of anti-MBP T cells than in control PBS-injected rats indicated that the visual system of the T cell-treated rats was much less affected by the secondary degeneration. The neuroprotective effect of the anti-MBP T cells on RGC survival and on the number of conducting axons after optic nerve injury was indeed manifested in an improved overall functional activity of the visual system.

These results further substantiate the notion that autoimmunity can be beneficial (Cohen, 1992), at least in CNS injuries, and suggest that although potentially pathogenic, autoimmune T cells can help to maintain tissue homeostasis and repair following trauma. It is possible that the autoimmune response triggered by injury is beneficial and is tightly regulated by self-tolerance mechanisms, leading to autoimmune disease only when its regulation is impaired. Pathological autoimmune diseases develop in only 3% of the population, despite the presence of self-reactive lymphocyte populations in all individuals (Burns et al., 1983; Schluesener and Wekerle, 1985; Martin et al., 1990; Pette et al., 1990). Mechanisms that terminate immune responses are important in self tolerance, where lymphocytes capable of recognizing self antigens are generated constantly, yet normal individuals remain unresponsive to their own antigens (Van Parijs and Abbas, 1998). Recent studies have demonstrated, however, that autoimmunity is awakened in response to CNS injury; T cells isolated from rats subjected to contusive injury of the spinal cord are capable of inducing EAE when transferred to naïve animals (Popovich et al., 1996). The spontaneous T cell response does not, however, exert enough protection to cause significant improvement after CNS injury. This might be attributable to immune privilege, which accounts for the inefficient and restricted communication between the CNS and the immune system. However, appropriate modulation of the immune responses at a site of CNS injury can promote protection and recovery of the CNS. We previously demonstrated that the neuroprotection induced by autoimmune T cells is similar whether the T cells are strongly or weakly encephalitogenic (Moalem et al., 1999a). Thus, the therapeutic use of non-encephalitogenic autoimmune T cells in the injured CNS might prove effective in reducing secondary damage, thereby preserving neuronal function.

Acknowledgements

We thank Shirley Smith for editorial assistance, Phil Taylor for statistical assistance, and Igor Friedmann for help with graphics. Dr. Dvora Teitelbaum generously donated guinea pig MBP. I.R.C. is the incumbent of the Mauerberger Chair in Immunology. M.S. holds the Maurice and Ilse Katz Professorial Chair in Neuroimmunology. The work is supported by a grant from the US army awarded to M.S., and a grant from the Alan Brown Foundation for Spinal Cord Injury awarded to M.S.

References

Ben Nun, A., Cohen, I.R., 1982. Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. J. Immunol. 129, 303–308.

Ben Nun, A., Wekerle, H., Cohen, I.R., 1981. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur. J. Immunol. 11, 195–199.

Burns, J., Rosenzweig, A., Zweiman, B., Lisak, R.P., 1983. Isolation of myelin basic protein-reactive T-cell lines from normal human blood. Cell. Immunol. 81, 435–440.

Cohen, I.R., 1992. The cognitive paradigm and the immunological homunculus. Immunol. Today 13, 490–494.

Dorfman, L.J., Gaynon, M., Ceranski, J., Louis, A.A., Howard, J.E., 1987. Visual electrical evoked potentials: evaluation of ocular injuries. Neurology 37, 123–128.

Duvdevani, R., Rosner, M., Belkin, M., Sautter, J., Sabel, B.A., Schwartz, M., 1990. Graded crush of the rat optic nerve as a brain injury model: combining electrophysiological, histological and behavioral outcome. Restor. Neurol. Neurosci. 2, 31–38.

Elias, D., Reshef, T., Birk, O.S., van der Zee, R., Walker, M.D., Cohen, I.R., 1991. Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65-kDa heat shock protein. Proc. Natl. Acad. Sci. USA 88, 3088–3091.

Faden, A.I., 1993. Experimental neurobiology of central nervous system trauma. Crit. Rev. Neurobiol. 7, 175–186.

Faden, A.I., Salzman, S., 1992. Pharmacological strategies in CNS trauma. Trends Pharmacol. Sci. 13, 29–35.

Gold, R., Hartung, H.P., Lassmann, H., 1997. T-cell apoptosis in autoimmune diseases: termination of inflammation in the nervous system and other sites with specialized immune-defense mechanisms. Trends Neurosci. 20, 399–404.

Hickey, W.F., Hsu, B.L., Kimura, H., 1991. T-lymphocyte entry into the central nervous system. J. Neurosci. Res. 28, 254–260.

Hirschberg, D.L., Moalem, G., He, J., Mor, F., Cohen, I.R., Schwartz, M., 1998. Accumulation of passively transferred primed T cells independently of their antigen specificity following central nervous system trauma. J. Neuroimmunol. 89, 88–96.

Hirshfeld, H., Teitelbaum, D., Arnon, R., Sela, M., 1970. Basic encephalitogenic protein: a simplified purification on sulfoethyl-Sephadex. FEBS Lett. 7, 317.

Hovda, D.A., Yoshino, A., Kawamata, T., Katayama, Y., Becker, D.P., 1991. Diffuse prolonged depression of cerebral oxidative metabolism following concussive brain injury in the rat: a cytochrome oxidase histochemistry study. Brain Res. 567, 1–10.

Liu, D., Yang, R., Yan, X., McAdoo, D.J., 1994. Hydroxyl radicals generated in vivo kill neurons in the rat spinal cord: electrophysiological, histological, and neurochemical results. J. Neurochem. 62, 37–44.

Lynch, D.R., Dawson, T.M., 1994. Secondary mechanisms in neuronal trauma. Curr. Opin. Neurol. 7, 510–516.

Martin, R., Jaraquemada, D., Flerlage, M., Richert, J., Whitaker, J., Long, E.O. et al., 1990. Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. J. Immunol. 145, 540–548.

McIntosh, T.K., 1993. Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review. J. Neurotrauma 10, 215–261.

Moalem, G., Leibowitz-Amit, R., Yoles, E., Mor, F., Cohen, I.R., Schwartz, M., 1999a. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nat. Med. 5, 49–55.

Moalem, G., Monsonego, A., Shani, Y., Cohen, I.R., Schwartz, M., 1999b. Differential T cell response in central and peripheral nerve injury: Connection with immune privilege. FASEB J. 13, 46–59.

Pette, M., Fujita, K., Wilkinson, D., Altmann, D.M., Trowsdale, J., Giegerich, G. et al., 1990. Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and healthy donors. Proc. Natl. Acad. Sci. USA 87, 7968–7972.

Popovich, P.G., Stokes, B.T., Whitacre, C.C., 1996. Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. J. Neurosci. Res. 45, 349–363.

Ramon y Cajal, S., 1959. In: May, R.M. (Ed.), Degeneration and Regeneration of the Nervous System,, Hafner, New-York.

Schluesener, H.J., Wekerle, H., 1985. Autoaggressive T lymphocyte lines recognizing the encephalitogenic region of myelin basic protein: in vitro selection from unprimed rat T lymphocyte populations. J. Immunol. 135, 3128–3133.

Smith, D.H., Casey, K., McIntosh, T.K., 1995. Pharmacologic therapy for traumatic brain injury: experimental approaches. New Horiz. 3, 562–572.

Spekreijse, H., Apkarian, P., 1986. The use of a system analysis approach to electrodiagnostic (ERG and VEP) assessment. Vision Res. 26, 195–219.

Streilein, J.W., 1995. Unraveling immune privilege. Science 270, 1158–1159.

Stys, P.K., Ransom, B.R., Waxman, S.G., 1991. Compound action potential of nerve recorded by suction electrode: a theoretical and experimental analysis. Brain Res. 546, 18–32.

Van Parijs, L., Abbas, A.K., 1998. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. Science 280, 243–248.

Yoles, E., Belkin, M., Schwartz, M., 1996. HU-211, a nonpsychotropic cannabinoid, produces short- and long-term neuroprotection after optic nerve axotomy. J. Neurotrauma 13, 49–57.

Yoles, E., Schwartz, M., 1998. Degeneration of spared axons following partial white matter lesion: Implications for optic nerve neuropathies. Exp. Neurol. 153, 1–7.

Yoles, E., Zalish, M., Lavie, V., Duvdevani, R., Ben Bassat, S., Schwartz, M., 1992. GM1 reduces injury-induced metabolic deficits and degeneration in the rat optic nerve. Invest. Ophthalmol. Vis. Sci. 33, 3586–3591.

Yoshino, A., Hovda, D.A., Kawamata, T., Katayama, Y., Becker, D.P., 1991. Dynamic changes in local cerebral glucose utilization following cerebral concussion in rats: evidence of a hyper- and subsequent hypometabolic state. Brain Res. 561, 106–119.

Preparation of Manuscripts

(continued from inside front cover)

Illustrations: Line drawings (including graphs) should be drawn in black ink on white paper or on tracing paper with blue or faint grey rulings; graduation will not be reproduced. Lettering should be large enough to permit photographic reduction. If figures are not to be reduced their format should not exceed 13.0×20 cm (the typing area of the Journal). Photographs (or half-tone illustrations) must be of good quality, submitted as black and white prints on glossy paper, and have as much contrast as possible. The degree of reduction will be determined by the publisher, but in general it should be assumed that the same degree of reduction will be applied to all figures in the same paper. For photomicrographs, where possible, a scale should appear on the photograph. Color plates will be reproduced at the expense of the author(s). Illustrations should be clearly marked on the reverse side with the number, orientation (top) and author's name; a soft pencil or preferably a felt-tipped pen should be used for marking photographs. The illustrations should be numbered with Arabic numerals. The legends should be typed separately.

Tables: Tables should be compiled on separate sheets with double spacing, numbered with Arabic numerals and provided with a short descriptive title, a legend and/or footnotes identified by superscripts ^{a,b,c,}, etc.

References: These should be cited in the text by author(s)' name(s) and year of publication (Harvard System). When referring to a work of more than two authors, the name of the first author should be given, followed by et al. Examples: (Emery, 1969); (Roy and Dubowitz, 1970); (Craig et al., 1978). All references cited in the text should be given at the end of the paper, typed *with double spacing* and arranged in alphabetical order of first author. More than one paper from the same author(s) in the same year must be identified by the letters a, b, c, etc. placed after the year of publication. References must consist of names and initials of all authors, year, title of paper referred to, abbreviated title of periodicals, volume number, and first and last page numbers. Periodicals, books and edited books should accord with the following examples, respectively.

Matyszak, M.K., Perry, V.H. 1998. Bacillus Calmette-Guerin sequestered in the brain parenchyma escapes immune recognition. J. Neuroimmunol. 82, 73–80.

Bradshaw, J., 1986. Basic Experiments in Neuropsychology. Elsevier, Amsterdam, pp. 143–163.

Blalock, J.E., 1992. Production of peptide hormones and neurotransmitters by the immune system. In: Blalock, J.E. (Ed.), Neuroimmunoendocrinology, Chemical Immunology, Vol. 52, Karger, Basel, pp. 1-19.

Journal titles should be abbreviated according to the *List of Serial Title Word Abbreviations* (International Serials Data System, 20 rue Bachaumont, 75002 Paris, France. ISBN 2-904938-02-8). This Journal should be cited in the list of references as *J. Neuroimmunol.*

Electronic manuscripts: Electronic manuscripts have the advantage that there is no need for the rekeying of text, thereby avoiding the possibility of introducing errors and resulting in reliable and fast delivery of proofs. For the initial submission of manuscripts for consideration, hardcopies are sufficient. For the processing of *accepted papers*, electronic versions are preferred. After *final acceptance*, your disk plus one, final and exactly matching printed version should be submitted together. Double density (DD) or high density (HD) diskettes ($3\frac{1}{2}$ or $5\frac{1}{4}$) are acceptable. It is important that the file saved is in the native format of the wordprocessor program used. Label the disk with the name of the computer and wordprocessing package used, your name, and the name of the file on the disk. Further information may be obtained from the Publisher.

Proofs, Reprints and Page Charge

One set of proofs will be sent to the author(s) to be checked for typographical errors. *No alteration to the text, tables or legends will be accepted.* In case of two or more authors please indicate to whom the proofs should be sent. Fifty reprints are provided free of charge. Additional copies (minimum 100) may be ordered at prices shown on the reprint order form.

***Journal of Neuroimmunology* has no page charges**

Subscriptions and Advertisements

Publication information: Journal of Neuroimmunology (ISSN 0165-5728). For 2000, volumes 100–109 are scheduled for publication. Subscription prices are available upon request from the Publisher or from the Regional Sales Office nearest you or from this journal's website (http://www.elsevier.nl/locate/jneuroim). Further information is available on this journal and other Elsevier Science products through Elsevier's website (http://www.elsevier.com). Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Claims for missing issues should be made within six months of the date of dispatch.

Orders, claims, and product enquiries: please contact the Customer Support Department at the Regional Sales Office nearest you:
New York: Elsevier Science, P.O. Box 945, New York, NY 10159-0945, USA; Tel.: +1-212-6333730, [toll free number for North American customers: 1-888-4ES-INFO (437-4636)]; Fax: +1-212-6333680; E-mail: usinfo-f@elsevier.com
Amsterdam: Elsevier Science, P.O. Box 211, 1000 AE Amsterdam, The Netherlands; Tel.: +31-20-4853757; Fax: +31-20-4853432; E-mail: nlinfo-f@elsevier.nl
Tokyo: Elsevier Science K.K., 9-15 Higashi-Azabu 1-chome, Minato-ku, Tokyo 106-0044, Japan; Tel.: +81-3-55615033; Fax: +81-3-55615047; E-mail: info@elsevier.co.jp
Singapore: Elsevier Science, No. 1 Temasek Avenue, #17-01 Millenia Tower, Singapore 039192; Tel.: +65-434-3727; Fax: +65-337-2230; E-mail asiainfo@elsevier.com.sg
Rio de Janeiro: Elsevier Science, Rua Sete de Setembro 111/16 Andar, 20050-002 Centro, Rio de Janerio–RJ, Brazil; Tel.: +55-21-5095340; Fax: +55-21-5071991; E-mail; elsevier@campus.com.br [Note (Latin America): for orders, claims and help desk information, please contact the Regional Sales Office in New York as listed above]

The *Journal of Neuroimmunology* is the official Journal of the International Society for Neuroimmunology. Information regarding the subscription rate available to members can be obtained from the Publisher or Dr. C.J. Lucas, TNO Prevention and Health, Department of Immunology and Infectious Diseases, Zernikedreef 9, 2333 CK Leiden, The Netherlands
Enquiries concerning manuscripts and proofs: questions arising after acceptance of the manuscript, especially those relating to proofs, should be directed to *Journal of Neuroimmunology*, Elsevier Science Ireland Ltd., Elsevier House, Brookvale Plaza, East Park, Shannon, Co. Clare, Ireland; Tel.: +353-61-709600; Fax: +353-61-709100.

Advertising information: Advertising orders and enquiries can be sent to: *USA, Canada and South America:* Mr Tino DeCarlo, The Advertising Department, Elsevier Science Inc., 655 Avenue of the Americas, New York, NY 10010-5107, USA; Tel.: +1-212-6333815; Fax: +1-212-6333820; E-mail: t.decarlo@elsevier.com. *Japan:* The Advertising Department, Elsevier Science K.K., 9-15 Higashi-Azabu 1-chome, Minato-ku, Tokyo 106-0044, Japan; Tel.: +81-3-5561-5033; Fax: +81-3-55615047. *Europe and the rest of the world:* Rachel Leveson-Gower, The Advertising Department, Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK; Tel.: +44-1865-843565; Fax: +44-1865-843976; E-mail: r.leveson-gower@elsevier.co.uk

Printed in The Netherlands